STUDIES IN MENINGOCOCCUS INFECTIONS.*

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BACTERIOLOGY.

DURING the winter and spring of the last two years (1905, 1906) there appeared in various parts of the city of Chicago, a number of cases of cerebrospinal meningitis. For the most part the cases were distinctly sporadic and such cases coming to the Cook County and Presbyterian Hospitals of this city afforded an opportunity for this study.

The chief points obtained from the bacteriologic examination are given in tabular form (Table 1). Of 11 cases, 10 occurred in men. The age varied from 3 to 32, six being between 10 and 20 years. For the most part they were foreigners living in the poorer parts of the city. Four of the 11 died. Diagnosis was made in all cases by lumbar puncture and the meningococcus of the Weichselbaum type isolated in every case. In smears made from the cerebrospinal fluid it was found in all cases but one, and this was a very mild case. Without exception the cerebrospinal fluid was under pressure and was turbid. Upon standing from 15 to 30 minutes a delicate clot of fibrin formed as a rule more abundant in the severer cases than in the milder ones, and also more abundant early in the disease than later. The fluid after the cells and fibrin were centrifugated out was usually clear and colorless. In one case it had a distinctly greenish tinge. This was during the third week and occurred in a mild case. Flakes of fibrin, white or yellowish in color, were occasionally obtained but never any blood clots. The fluid when a free flow was obtained, always became practically colorless after the blood due to the puncture had been washed away. Uncoagulated blood was not present in any instance. Hemolysis in the fluid was never encountered. Differential counts of the leucocytes were not made but the polynuclear leucocytes were by far the predominating cell, there being always a few mononuclears and large cells of the endothelial type. In one case examined late in the disease (fourth week) the latter cells were very numerous.

The diplococci were found both inside and outside the polynuclear leucocytes and never inside any other cell. The number varied considerably, being on the whole more numerous in the severe cases than in the milder. There was considerable variation in the numbers of cocci in different cases and in the same case from time to time. As a rule, the less severe the disease the greater the number of cocci found inside the cells, but this is only generally true and exceptions occurred. During convalescence especially, the cocci were nearly always found more completely englobed by the cells than earlier in the disease.

The organisms corresponded in every instance to the Weichselbaum type of meningococcus. All were Gram-negative and showed no appreciable variations in

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TABLE 1.

BACTERIOLOGY OF CASES OF EPIDEMIC CEREBROSPINAL MENINGITIS.

No.	Sex	Age	Nationality	Meningococci in Cerebrospinal Fluid		Meningococci on Nasal Mucosa		Blood	Eye	Agglutination	Termination
				Smears	Cultures	Smears	Culture				
1	M	19	Italian	++Chiefly outside leucocytes	+	Gram-negative diplococci	30%	—	....	—	Death
2	M	19	Italian	++Mostly outside leucocytes	+	Gram-negative diplococci	o	o	....	+1:50	Recovery
3	M	A[1]	Italian	+	+	Gram-negative diplococci	o	o	....	—	Death
4	M	16	Italian	+	+	Gram-negative diplococci	o	o	....	+1:50	Recovery
5	M	26	Italian	O	+	No Gram-negative diplococci	o	o	....	+1:100	Recovery
6	M	32	Irish	+Chiefly inside leucocytes	+	Not examined	Not examined	—	....	—	Recovery
7	M	10	German	++Mostly outside leucocytes	+	Gram-negative diplococci	+90%	+	....	+1:50	Death
8	M	3	German	++Numerous; chiefly inside the leucocytes	+	Gram-negative diplococci	+90%	+	Purulent conjunctivitis	+1:100	Recovery
9	M	30	Bulgarian	+Largely inside leucocytes	+	Gram-negative diplococci	+ 5%	o	Men.+, nearly pure Conjunctivitis. Meningococci negative	+1:500	Recovery
10	F	18	German	Not examined	+	Not examined	Not examined	o	....	+1:50	Death
11	M	13	American	+ Few cocci outside of the leucocytes	+	Not examined	Not examined	o	....	+1:50	Recovery

[1] A = Adult.

this respect. For cultivating the cocci the spinal fluid was usually added to ascites broth (1:4) which was found suitable and reliable for this purpose. In 24 hours, growth is manifested by a marked tubidity. Broth to which a small quantity of defibrinated blood has been added also makes a good medium. Blood-agar, Löffler's blood serum and calcium broth are all satisfactory. The cultures must be transferred every three or four days or preferably every other day. At the end of four or five days they often die. Cultivation of several strains for over four months, transfers being made on Löffler's blood-serum every three or four days and often more frequently, did not show any appreciable alteration in the cultural or morphological characteristic except, perhaps, a little more vigorous growth. For plating ascites-glucose-agar (1:4) and blood agar (10 drops in 5 c.c. of glucose-agar) are good media. The colonies in 24 hours are 0.5 to 1 mm. in diameter with sometimes larger surface colonies. They are of a delicate bluish gray and do not hemolyze. When grown on plates they give off a peculiar odor difficult to describe but suggesting moldy earth. This is always obtained but does not appear to be characteristic. On blood serum one frequently sees a very profuse growth like a giant colony 2 or 3 mm. in diameter decidedly raised above the surface. This may be one large colony or may be formed by the fusion of several colonies. It appears to be quite characteristic of the meningococcus. The growth especially on blood serum is often tenacious and when suspended in salt solution or water appears as flakes and threads which may be broken up by vigorous agitation. Strains vary in this property, it being evident in all, but in many it is much more conspicuous than in others. It differs decidedly in this respect from *M. catarrhalis*, whose growth is quite brittle, is readily broken up, and never tends to form flakes when suspended from blood serum in salt solution.

Examination was made of the nasal secretion in eight cases. In smears made directly from the mucosa, Gram-negative diplococci morphologically suggestive of the meningococcus were observed in seven of the eight cases. In four cases meningococci were isolated on blood-agar or ascites-agar plates and in two of these (Cases 7 and 8) they formed approximately 90 per cent of all organisms present in the plates. In Case 1 they formed about 30 per cent and in Case 9 about 5 per cent. In the smears made from the mucus the Gram-negative diplococci were very numerous, and it appears, from a comparison of the plates and the smears, that often a large number of the cocci occurred which were dead or did not develop. There is little doubt that the Gram-negative organisms seen in nearly all the smears were meningococci, though, so far as one could tell from their morphology, they may have been *M. catarrhalis*. Among other organisms obtained, besides the commonly found Gram-positive diplococci of the pneumococcus type may be mentioned the occurrence in five out of the eight cases examined, of hemophilic bacteria resembling the organisms found in measles, whooping-cough, bronchitis, influenza, and other infectious diseases. In some of the cases they were very abundant and, it is possible, played a part in producing the nasal symptoms.

In two cases (Cases 8 and 9) a conjunctivitis existed early in the disease. In Case 8 there was a purulent discharge, smears of which showed some Gram-negative diplococci. Cultures gave a nearly pure growth of typical meningococci. The conjunctivitis was not severe and in a few days (under boric acid irrigation) cleared up, leaving no lesion. In Case 9 smears and cultures from the conjunctiva showed almost pure growth of a bacillus of the diphtheria group. No meningococci were present. This infection likewise cleared up in a few days.

Blood cultures were made in 9 cases with two positive results. This is about 22 per cent or about the same as found by Elser[1] in New York. In both of these cases (7 and 8) positive results were obtained within the first four days of the disease. Case 7 died on the fifth day and Case 8 recovered. In some of the cases cultures were not obtained until after the first week. I feel quite confident that if cultures of the blood, using large quantities, were made very shortly after the onset of the attack and at frequent intervals thereafter the percentage of positive results would be much higher. In Cases 2 and 9 both severe, though both ultimately recovered after a long period, cultures were made at three different times at intervals of a few days, always with negative results. That the presence of the meningococcus in the blood does not necessarily mean a fatal result is shown by the result in Case 8.

Herpes was present in nearly all the cases. In two, several cultures were made from the contents of the vesicles at different stages. Staphylococci were always obtained but no meningococci.

Case 8 shows the remarkable extent to which invasion of the body by meningococci is possible and recovery ensues. The organisms in this case were cultivated from the eyes, nose, blood, and cerebrospinal fluid. On the nasal mucosa they were very numerous. From the blood an abundant growth was obtained by introducing 1 c.c. in ascites broth. During the third week of the disease a double otitis media developed. Cultures and smears were made from the discharge on the first and fifth days after it appeared and at the same time cultures were made from the nasal mucosa. At this time a diphtheria-like bacillus was obtained from the nose and a mixed growth of this bacillus and streptococci found in both ears. No meningococci were found. This is evidently therefore an instance in which a primary meningococcus infection furnished suitable conditions for the secondary invasion of other organisms, a process so commonly seen in other acute infections.

The manner and order in which the meningococcus gains access to the various tissues of the body is a problem of importance but one difficult to solve. That many cases of meningitis are associated with bacteria in the blood is established. Since it is an organism much more difficult to cultivate than the typhoid bacillus or pneumococcus, we can hardly expect to obtain the meningococcus from the blood in all the cases where it is present. Other evidence of the invasion of the body by the meningococcus is localization of the organism in the pericardium, in various joints, and in other places. In this series there occurred a purulent pericarditis in one case. Pure meningococcemia with no definite localization elsewhere than in the meninges is rare; a few cases are reported. Cases have been described also in which the organism has been recovered from the blood and from joint lesions some time before the appearance of meningeal symptoms, thus indicating a primary blood infection with secondary localization in the meninges. These observations naturally bring

[1] *Jour. Med. Res.*, 1905, 14, p. 89.

up questions as to the mode of entry of the meningococcus into the system.

In view of the relatively frequent occurrence of the meningococcus on the nasal mucosa this locality may be an important starting-point in its invasion of the body. As was first suggested by Weichselbaum, the frequent preceding and coincident acute rhinitis makes this view seem reasonable, the inflamed mucosa allowing the cocci to pass directly into the blood vessels and lymph spaces. There is also the possibility, as Westenhoeffer has pointed out, that the throat and tonsils may be portals of entry since they not infrequently are involved early in the disease. The meningococcus is often found in abundance in the throats of patients. Probably the mucosa of the whole respiratory tract should be looked upon as at times affording a suitable point of entrance for the meningococcus. It is improbable that the gastro-intestinal tract is a portal of entry for the cocci because they are so sensitive to acids.

It is commonly supposed that the organisms, after passing through the mucosa, enter the lymphatics and make their way directly through the base of the skull to the meninges. However, it does not necessarily follow that because the point of entrance and the localization of infection are near each other the organism reaches the latter place by the shortest possible route. The predilection for localization upon the meninges may be analogous to what occurs in other infections. Thus, intravenous injection of cholera bacilli into animals is followed by a localization of the infection in the small intestine. Dysentery becomes localized in the large intestine. Gonococci localize generally upon serous surfaces, especially of the joints. Tubercle bacilli enter through the respiratory or intestinal mucosa and often localize in some remote part of the body, causing no lesion at or near the place of entry. So meningococci, when once in the blood or lymph, may be carried throughout the whole body and only secondarily, because of strong affinity for the meninges, settle there. Occasionally, as in other diseases, an anomalous localization occurs, such as in a joint; or localization may fail to take place and a meningococcemia results.

While the anatomical relations of the nasal mucosa and the meninges may play some part in determining the site of the lesion, an even more important factor would seem to be the peculiar affinity of the organism

for the meninges. The nature of this affinity is not known, but it conforms to a principle common in infectious diseases and emphasizes that the chemistry of the structures may be of greater importance than anatomical relations in determining the localization of an infective process.

The discussion of the bacteriology of meningitis naturally raises the question of the manner in which the organisms are distributed and how sporadic cases arise. The large numbers of cocci in the secretions of the nose and throat of patients, show how easily the cocci may be transferred by coughing, sneezing, breathing, etc. Their occurrence in the nose and throat of persons associated with meningitic patients which has now been demonstrated repeatedly, is sufficient evidence that the cocci are easily transferred, and emphasizes the importance of careful isolation of cases and proper disinfection of the throat and nasal cavities of persons closely associated with patients.

Sporadic cases, however, present problems of their own. They are identical with those in epidemics, the organisms from both groups corresponding in every respect. The meningococcus is so delicate that it does not seem possible that it can live over, at the most, a few days outside of the human body. Animals, so far as we know, are naturally immune and do not harbor the coccus. There are, therefore, only two possibilities to explain sporadic cases. The first is that the cocci are carried from person to person without causing symptoms until, perhaps, after many transfers an individual is encountered in whom the conditions are favorable for their development and entrance into the system. If this be true, one would expect to find meningococci, occasionally at least, in the throats and noses of individuals, normal or abnormal. This, however, does not appear to be true from the data at hand. In over 200 examinations, made by the writer, of the sputum and throats of more than 150 individuals, by means of the blood-agar plate method, the meningococcus was never found. Others have made careful bacteriological examinations of the throat in health and disease but report no meningococci. Hasslauer[1] studied 192 cases with especial reference to the meningococcus and concludes that this organism occurs only in the throat of men-

[1] *Centralbl. f. Bakt.*, 1906, 41, p. 633.

ingitic patients and of persons in their immediate vicinity. However, this does not prove absolutely that the meningococcus does not occur occasionally in the normal throat, for if only occasionally present in few numbers it might easily elude detection. It does show, however, that the meningococcus, unlike the pneumococcus and streptococcus, if it occurs at all, must do so infrequently and then probably in small numbers; otherwise it would surely have been detected.

The other possibility, which is remote, is that closely related organisms, commonly found on the respiratory mucosa, may be modified under certain conditions and acquire all the properties of the meningococcus. There is a group of Gram-negative diplococci, including *M. catarrhalis*, found in the nose and throat, some of which closely resemble the meningococcus but yet possess well-marked points of difference in cultural characteristics and especially in agglutinating properties. Such transition has not been observed, and is as yet a theoretical possibility.

PROPERTIES OF THE BLOOD AND SERUM IN MENINGITIS.

Agglutination.—Agglutination tests were made in eight cases. In all a positive result was obtained in dilutions of 1:50. Both microscopic and macroscopic methods were used. The highest dilution at which agglutination occurred was 1:500 (Case 9). This was at the beginning of the fourth week of the disease. At the end of the first week the serum agglutinated strongly at 1:25, at 1:50 slightly; in the eighth week the agglutination occurred at 1:200 and not at 1:500. This case recovered after a protracted course. In two other cases the agglutination occurred at a dilution of 1:100. A distinct reaction was not obtained in the other cases higher than at 1:50.

The agglutination test in Case 8 gave negative result on the fifth day after the first evidence of any symptoms, or the third day after distinct meningeal symptoms had appeared; it was obtained with an organism isolated from another case. Two days later agglutination appeared at a dilution of 1:25 with this same organism and also at the same dilution with meningococci isolated from the blood, cerebrospinal fluid, nose, and conjunctiva of the case itself. Later the serum agglutinated at a dilution of 1:100. In two other cases agglutination

was obtained on the seventh day. From the above data we see that agglutination is obtainable at the end of the first week, approximately.

The meningococcus agglutinins are thermostable bodies resisting, with little diminution in their activity, a temperature of 65° C. for one hour.

The cerebrospinal fluid does not appear to contain the agglutinins in an appreciable amount. In Case 9 the pure fluid gave practically no evidence of clumping, while the serum of the patient removed at the same time produced marked agglutination at 1:50 or higher. This test was made at two different times and both homologous and heterologous strains of organisms were used. I have also looked for clumping of the cocci in the fluid as it comes from the canal in cases in which the serum gave a positive test at 1:50 or higher, but always with negative results.

A rabbit was immunized with a typical meningococcus isolated from the spinal fluid of Case 6. Subcutaneous inoculations were first used, followed by intraperitoneal and intravenous. The animal decreased in weight and lost much of its hair; it became immune to large doses of meningococci, easily resisting 10 times the dose that at first made the animal quite ill. A number of strains isolated from various sources were tested and the result is given in Table 2. The

TABLE 2.

AGGLUTINATION OF MENINGOCOCCI BY IMMUNE RABBIT SERUM.

Source of Organism	1:20	1:50	1:100	1:200	1:500	Salt Solution
1. Cerebrospinal fluid (Case 9)	++	+	o	o	o	o
2 Nasal Mucosa (Case 9)	++	++	o	o	o	o
3 Cerebrospinal fluid (Case 6)	++	++	o	o	o	o
4 " " " (Case 8)	++	+	o	o	o	o
5 Nasal Mucosa (Case 7)	+++	++	o	o	o	o
6 Conjunctiva (Case 8)	++	+	o	o	o	o
7 Blood (Case 8)	+++	++	o	o	o	o
8 *M. catarrhalis*	++	o	o	o	o	o

homologous organism was agglutinated at a slightly higher dilution than the other organisms, but the difference is not great. The serum of patients also shows no special difference in its agglutinating power over different strains.

M. catarrhalis (isolated from a case of whooping-cough) showed less clumping than the meningococci. The controls with normal rabbit serum gave negative results or only very slight clumping at 1:10.

It may be stated that all the meningococci isolated from the blood, nose, and eyes of the cases reported were tested either with immune rabbit serum or with serum of patients, and that all, without exception, reacted positively. They are therefore considered typical meningococci.

Antimeningococcal properties of blood and serum.—In a previous paper[1] it was shown that meningococci, when introduced into normal defibrinated blood, as a rule would not multiply. In some normal bloods, after an initial drop, the cocci multiplied rapidly, thus indicating interesting individual variations. In the blood of several meningitic cases tested at varying times from the tenth day of the disease to the seventh week, the meningococci without exception were rapidly killed, the plates usually being sterile at the end of three hours. It was also found that in the serum of both normal persons and meningitic cases the meningococci rapidly die, but that the meningococcidal effect of the meningitic serum is greater than that of the normal. Furthermore, the destructive effect of the serum is much greater than that of normal salt solution so that, while the cocci tend to die out rapidly as a result of autolytic processes, there can be no doubt but that serum exerts a distinct lytic effect on meningococci, and this effect is greater in meningitic serum than in normal.

In Table 3, the meningococcidal effect of meningitic serum in the fourth week is given. The serum was tested by the plate method in

TABLE 3.

EFFECT OF MENINGITIC SERUM ON MENINGOCOCCI.

		At once	3 Hrs.	6 Hrs.	24 Hrs.
1	Nor. Ser. 0.4	6,000	0	0	0
2	" " 0.3+asc. br. 0.1	6,000	51	0	0
3	" " 0.2+ " " 0.2	4,600	166	176	500
4	" " 0.1+ " " 0.3	5,000	222	140	9,000
5	Men. Ser. (4 W.) 0.4	5,000	0	0	0
6	" " " 0.3+Asc. br. 0.1	4,000	16	0	0
7	" " " 0.2+ " " 0.2	5,500	67	3	0
8	" " " 0.1+ " " 0.3	6,000	220	88	2,500
9	Ascites Broth	6,000	4,500	7,000	10,000

two cases early in the disease, one on the fifth day and the other on the sixth. In both cases (7 and 8) the organism was cultivated from the same blood from which the serum was obtained for the experiment. In each case the meningitic serum was slightly less bactericidal than

[1] *Jour. Infect. Dis.*, 1905, 2, p. 602.

the normal serum; but the difference is so slight that one is hardly justified in considering it of great significance. The agglutination test in one case gave negative results at the time; in the other case the test was not made until two days later when it was positive at 1:25.[1] In Case 7, also on the fifth day, the meningococcidal effect of the defibrinated blood, in which meningococci were found by cultures, was tested and in this case the cocci grew. This was only a short time before death; the leucocyte count was 22,000. We see then that late in the attack the serum acquires definite meningococcidal properties. The subject of phagocytosis of meningococci is taken up elsewhere and here it is sufficient to state that the cocci are readily taken up by the leucocytes in defibrinated blood and very soon undergo rapid disintegration within the cells.

From some early experiments it did not appear that the meningococcidal property of the blood ran parallel with the leucocytosis, but was found to be increased later in the disease when the leucocytic count was practically normal. And, as stated, meningococci grew in defibrinated blood in one case when the leucocytosis was high (22,000). Samples of blood containing varying quantities of leucocytes were obtained by centrifuging and using the top and bottom layers, the former containing many leucocytes and the latter very few. This blood was now heated to 46° C. for 10 minutes, so as to destroy phagocytosis but not to injure the action of complement. The results are

TABLE 4.

EFFECT OF LEUCOCYTES ON MENINGOCOCCI.

		Leucocyte Count	At once	3 Hrs.	6 Hrs.	24 Hrs.
1	Defib. men. blood 0.4 c.c.....	30,000	1,600	0	0	0
2	" " " 0.4	11,200	6,000	0	0	0
3	" " " 0.4	7,100	1,500	0	0	0
4	" " " 0.4	4,000	1,000	0	0	0
5	" " " 0.4	600	5,400	4	0	0
6	" " " 0.4	4,000	5,000	2	0	0
	(Heated to 46° C. 10 min.)					
7	" normal blood 0.4 c.c.....	4,400	1,100	37	1,800	15,000+
8	" " " 0.4 c.c.....	4,400	2,000	111	10,000	6,000
	(Heated to 46° C. 10 min.)					

[1] The point may be raised that the apparent meningococcidal action, as shown by the plate method, is due to the agglutination of the organisms. I have no doubt but that in agglutinating sera the clumping may explain at least part of the diminution in the number of colonies appearing on the plates at various intervals. However, this does not alter the fact that the bacteria are killed, as shown by the result after several hours, and it is immaterial to us in this present consideration of the question whether part of the bactericidal action, as shown by this method, is due to agglutinins or all due to other factors, such as complement action or an enzymotic process.

given in Table 4, and we see that the number of leucocytes in the blood has little if any effect on the organisms so far as results on plates indicate. In one experiment blood containing 600 leucocytes was just as fatal as one containing 30,000.[1] In a normal blood, in which the cocci readily multiplied, the killing of the leucocytes by heat made no difference in the rapidity of multiplication. It is difficult to understand why the effect of phagocytosis is not more apparent; it is possible that it is overshadowed by the stronger action of the serum.

As shown previously meningococci do not grow in cerebrospinal fluid, either normal or meningitic. *In vitro* one cannot obtain the same conditions as are present in the spinal canal. After leaving the canal fibrinogen, for instance, is soon transformed into fibrin, i. e., changed from a soluble to an insoluble compound. Such changes in the properties may explain why the cocci grow profusely in the canal and rapidly die in the test tube. Serum may be added to cerebrospinal fluid or salt solution in any proportion; the organisms nevertheless die rapidly. If red corpuscles, however, are added to the fluid an excellent medium is obtained. This indicates that the blood in the cerebrospinal fluid may facilitate greatly the growth of meningococci.

There are then several factors concerned in the development of meningococci. In the first place the organism is extremely sensitive and, as we have seen, dies in salt solution in a few hours. Flexner[2] recently concludes that this is largely due to autolysis. In serum the cocci may be observed directly to undergo a rapid disintegration. While this process of autolysis probably goes on in serum, as it does in salt solution, there is also another factor which makes the serum much more unfavorable than salt solution. This may be an amboceptor-complement action and the increased meningococcidal power of meningitic serum may be due to an increase in the amboceptor during the course of the disease. Phagocytosis we cannot doubt also plays a part in the destruction even though it is not clearly brought out by the plate method. Opposed to these destructive processes there are other conditions in blood directly favoring growth, probably the most

[1] The amount of serum in the tubes was as nearly as possible the same.

[2] *Jour. Exp. Med.*, 1907, 9, p. 105.

important of which is the presence of red cells or hemoglobin. Of course other proteids of the blood may favor growth also. Serum, for instance, which in high concentrations is deadly, favors growth when sufficiently diluted, as shown by adding serum to plain broth, in which meningococci do not grow at all or very poorly; yet when the serum is diluted several times by the broth a favorable medium is formed. Here the antagonistic effects of the serum when concentrated is superseded by its nutritive properties.

Thus we find two sets of factors, one favoring the multiplication of meningococci, the other destructive. Infection therefore with this organism and the character of its subsequent course must depend upon the relation which these two sets of factors bear to each other in the various tissues in the body, and especially in the blood and the cerebrospinal fluid. It is evident from the data given upon the effect of blood on meningococci that in this substance these factors are almost in a state of equilibrium. Sometimes the cocci grow and sometimes they do not. No great change is required to upset this adjustment. There are no definite data at hand bearing upon changes in these factors that may precede an attack of meningitis. During the course of the disease, however, we note a definite change in favor of the host and against the invading microbe, namely an increased meningococcidal property of the serum, due perhaps to an increase in amboceptor, and, as is shown subsequently, in an increase in the opsonic content of meningitic serum as compared with normal serum. When we consider the delicate balance of various factors in normal blood it is probably true that changes even so small as to be within the present limits of experimental error may be quite sufficient to bring about a destruction either of the host or of the microbe.

Phagocytosis.—As shown previously[1] meningococci are readily taken up *in vitro* by leucocytes in the presence of serum, but practically not at all by washed leucocytes or by leucocytes in the presence of normal serum heated to 60° C. for 30 minutes.. The number of cocci taken up by the leucocytes is roughly proportional to the amount of serum present as shown in Table 5.

In view of the fact that the opsonic content has been found to be increased in some acute infections one naturally would look for a

[1] *Jour. Infect. Dis.*, 1905, 2, p. 602.

TABLE 5.

PHAGOCYTOSIS IN NORMAL AND MENINGITIC SERA.

										Normal Serum	Men. Ser. 10th Day (Case 8)	Men. Ser. 2d Wk. (Case 9)
1	Washed Corp.	0.2 c.c.	+	Ser. 0.2	+	Susp. cocci.		*0.2...		6.1	8.9	7.6
2	" "	0.2 c.c.	+	" 0.1	+	" "		0.2...		2.9	6.1	4.5
3	" "	0.2 c.c.	+	" 0.05	+	" "		0.2...		2.6	4.7	3.1
4	" "	0.2 c.c.	+	" 0.025	+	" "		0.2...		1.6	2.4	2.1
5	" "	0.2 c.c.	+	" 0.0125	+	" "		0.2...		1.6	2.4	1.6
6	" "	0.2 c.c.	+	" 0.00625	+	" "		0.2...		0.4	1.0	0.9

* The organism used in this experiment was obtained from the cerebrospinal fluid of Case 8. Note the greater phagocytosis in the homologous serum. In each case the total quantity was brought up to 6 c.c. and the mixtures incubated at 37° C. for 10 minutes. The number of cocci given represents the average within 100 leucocytes.

similar increase in meningitis. In my previous paper I stated that by the method used no appreciable increase was observed in the opsonin in the few cases then studied.

Since then I have made a number of experiments using falling quantities of serum and testing the blood from time to time in individual cases. While many of the experiments were unsatisfactory—because of certain difficulties referred to later the results on the whole show somewhat greater phagocytosis in meningitic serum than in normal serum (Table 5). Other determinations did not show as great

TABLE 6.

PHAGOCYTOSIS OF MENINGOCOCCI IN MENINGITIC AND NORMAL SERA.

	Source of Organism	Meningitic Serum (Case 9) 8th Wk.	Meningitic Serum (Case 10) 4th Wk.	Normal Serum
1	Cer.-sp.-fluid (Case 10).....	4.6	4.4	2.2
2	" " " (Case 9).....	5.0	8.3	2.4
3	Blood (Case 8).....	5.8	7.7	4.3
4	Nasla mucosa (Case 7).....	9.8	13.8	9.7
5	Cer. sp. fluid (Case 11).....	4.4	5.1	4.9
6	" " " (Case 8).....	8.1	11.8	8.0
7	Conjunctiva (Case 8).....	14.0	16.5	7.5
8	Cer. Sp. fluid (Case 9).....	3.0	4.8	3.5
9	Nasal mucosa (Case 9).....	8.2	10.2	9.8
	TOTAL.....	62.9	82.6	52.3
	AVERAGE	7.0	9.2	5.8

a difference between normal and meningitic serum and others showed a greater. Table 6 gives the results obtained with normal serum and the serum of two meningitic patients, one of whom had been sick about four weeks and the other eight. Nine strains of meningococci from various sources were used. Some strains show a marked difference of phagocytosis in favor of the meningitic sera, others very

little or none at all. On the whole the serum from the patient in the fourth week shows a marked increase. If the numbers indicating the phagocytosis for each serum are added together and the average determined, the amount of phagocytosis is seen to be decidedly greater in the cases of the meningitic sera. The same suspensions of organisms were used for each serum so that the results are comparable. In order to obtain the exact course of the meningococco-opsonic index in meningitis daily observations should be made throughout the attack.

If normal cerebrospinal fluid is added to washed corpuscles no phagocytosis results, and the same seems true when the fluid of meningitic patients is used; at all events the amount of phagocytosis is very slight. The fluids from two cases gave practically the same result. In each instance the fluid before centrifugation contained many meningococci and leucocytes, some of which contained organisms. If counts were made of the phagocytosis *in vivo*, the count on the whole would be low, for many of the leucocytes are disintegrating and the cocci are few in numbers as a rule. We may conclude that normal cerebrospinal fluid has practically no opsonin and the meningitic fluid has a small but insignificant amount as compared with that in blood serum. It probably does not require much opsonin in order to give the phagocytosis that occurs in meningitic fluid, because cocci are exposed to the opsonin for a relatively long period of time under favorable conditions.

In regard to the susceptibility to phagocytosis of different strains of meningococci there does not appear to be a marked difference. They are susceptible to phagocytosis immediately after isolation from the spinal fluid and from the blood, and are apparently as freely taken up at this time as after cultivation (Table 6). All strains are susceptible, but the figures as given are not comparable because of the varying concentration of the suspensions. Since the organisms are so freely taken up, slight differences in susceptibility to phagocytosis, which may mean a difference in virulence, is difficult to detect. Strain 1, for instance, was an organism isolated from the cerebrospinal fluid of a fatal case and had been under cultivation only a few days. It was highly toxic for man as shown by subsequent subcutaneous injection. No. 8 was an organism isolated from the cerebrospinal

fluid of a very mild case and had been under cultivation for several months, being transferred to fresh media every two or three days. There seems to be little difference in the susceptibility of these two organisms to phagocytosis. One sometimes observes strains which apparently are not taken up or seemingly much less so than others. This I have found to be explained by the fact that many of the organisms of some cultures stain not at all or very poorly and irregularly, and consequently the counts are unreliable.

It was said that on the whole there was less phagocytosis observed in the spinal fluid of severe cases than of milder. This may mean a slight difference in virulence; but it may also be explained by a slight difference in opsonic content of the fluids, or possibly by difference in the activity of the leucocytes. In this respect the meningococcus is in marked contrast to the pneumococcus which, as shown by Rosenow,[1] is nearly or wholly insusceptible to phagocytosis when isolated from the blood of pneumonic patients. It is true that generally meningococcus meningitis is not so virulent or so fatal an infection as pneumococcus meningitis. In the latter the leucocytes contain practically no organisms though as a rule the pneumococci are extremely abundant in the fluid outside the cells. In three cases, the fluids of which I had an opportunity to examine and compare with that of meningococcus meningitis, the organisms in all were very much more numerous in the fluid than in any of the meningococcus infections studied, and practically no phagocytosis of pneumococci had occurred.

If serum is added, either to washed leucocytes in blood or to the washed leucocytes of freshly drawn meningitic fluid, the number of meningococci taken up is much increased. Two experiments with leucocytes from the spinal canal, to which serum was added showed a rise in one case from 1.1 to 3.1 and in the other from 1.2 to 8.0. From these results it would seem rational to inject human serum into the spinal canal of patients in order to increase the destructive action of the leucocytes.

Two cases were thus treated. The first case was one of only moderate severity running an ordinary course with intermittent fever. During the third week a spinal puncture was made, about 10 c.c. of a turbid fluid withdrawn and 8 c.c. of normal fresh human serum was

[1] *Jour. Infect. Dis.*, 1907, 4, p. 285.

injected in its place. There was no reaction and the patient made an uneventful recovery. One cannot say that the injection had any effect whatever, except that it did no harm.

The second case was very severe. The onset occurred with a chill and vomiting, and the temperature ranged from 104 to 106° F. There was present opisthotonus, marked rigidity of the neck, Kernig's sign, and delirium. Cultures from the nose and blood showed meningococci. The spinal fluid on the fourth day after onset was turbid, contained much fibrinogen and abundant typical meningococci chiefly outside the leucocytes. On the fifth day in the evening lumbar puncture was made and 35 c.c. of turbid fluid under considerable pressure were withdrawn and 10 c.c. of normal human fresh serum were then slowly introduced. The next morning, about 12 hours later, only 1 c.c. of turbid fluid could be obtained. There were present many leucocytes and they contained few diplococci within them. Five c.c. of fresh serum were again injected. The child gradually became worse and died on the seventh day. The serum did not have any apparent effect on the course of the disease. From the smears of the spinal fluid made 12 hours after the injection of the serum one could observe no appreciable increase in phagocytosis.

The difficulties in the way of this treatment are the failure of the serum to reach the meninges of the cord and brain and the large amount of fluid in the canal making the dilution so great that a small amount of serum could have little effect either on phagocytosis or on bacteriolysis.

Hemagglutinins and hemopsonins in meningitis.—In the phagocytosis experiments it was noted that a distinct clumping of corpuscles frequently occurred. A number of tests was made to determine if the agglutination of red corpuscles by meningitic sera was in any way characteristic.

The results show that while agglutination of red corpuscles from various sources is a common property of meningitic sera it is not specific, and sera from different cases behave differently toward the same red corpuscles. In three cases meningitic serum did not clump homologous corpuscles. It clumped some normal red corpuscles and not others. It may or may not clump meningitic red corpuscles or corpuscles from other infections as scarlet fever and pneumonia.

No changes were observed during the course of the disease or later in the agglutinative property toward various corpuscles.

The agglutinins are absorbed *only* by corpuscles which are agglutinated and may be completely removed from the serum by contact for a few hours. The agglutinin cannot be recovered by washing the clumped corpuscles in salt solution. The hemagglutinin and bacterial agglutinin present in meningitic sera were shown to be independent bodies by the fact that the serum freed from hemagglutinin by long contact with red corpuscles still retained unimpaired its power to agglutinate meningococci. Ascites broth in which meningococci are grown for varying periods (24 hours, 48 hours, one week, and two weeks) at no time show the slightest trace of hemagglutinin. Several strains of meningococci were used. The hemagglutinins are fairly stable bodies. Heating to 65° C. for 30 minutes diminishes the agglutinative power of serum but does not completely destroy it. Exposure to sunlight slowly destroys the property; but much more rapidly in the presence of eosin. The above data indicate that the hemagglutinins found in meningitic blood are not different from those occurring in normal blood; they possess no specific properties. My results agree in every respect with those of Hektoen[1] and others.

Phagocytosis of red corpuscles was observed in two meningitic sera. It was obtained by adding the sera to washed normal corpuscles and allowing to stand for one hour. The serum in one case (Case 8) was obtained on the seventh day of disease. In the other case (Case 11) the serum was obtained in the second week. The corpuscles were strongly agglutinated by the sera. Sera from two other cases of meningitis were repeatedly tested for hemopsonins always with negative results. They were strongly agglutinative for certain red corpuscles, but no phagocytosis of the corpuscles was observed at any time.

THE INJECTION OF MENINGOCOCCI INTO ANIMALS AND MAN.

It is well known that meningococci have only a small degree of virulence for the lower animals. Large doses of these organisms, either dead or alive, may be given to animals subcutaneously or intravenously with little effect; and it has been questioned whether or not the living organisms multiply at all after injection into the animals.

[1] *Jour. Infect. Dis.*, 1902, 4, p. 297.

I have injected 24-hour growths from two blood-serum slants directly into the veins of a small monkey with not the least effect. Flexner[1] has recently produced lesions in monkeys by intraspinal injections of meningococci, but he is not sure that multiplication, at least to any great extent, occurred. His results also show how insusceptible animals are to subcutaneous injections.

I have repeatedly injected rabbits subcutaneously with growths from five to ten tubes of blood serum with practically no effect. Intraperitoneal injections are more effective, but even here the lower animals can tolerate large doses of meningococci, as compared with many other organisms, as for instance virulent streptococci. Strains vary in their virulence to a certain extent, as Flexner has pointed out.

To show the result of successive inoculations into animals the following experiment was made. A typical meningococcus isolated from the cerebrospinal fluid of a non-fatal case was used.

Guinea-pig 1.[2]—Inoculated intraperitoneally with growth from one blood-serum slant. Dead in 12 hours. Peritoneal cavity contained a gelatinous non-purulent exudate in which meningococci were very numerous, leucocytes very few, and there was very little phagocytosis. Cultures from heart's blood and peritoneal exudate gave abundant meningococci.

Guinea-pig 2.—One-half c.c. of peritoneal exudate from Pig 1 was injected intraperitoneally. Next morning the animal was lively, showing no ill effects whatever.

Guinea-pig 3.—Growth from one blood-serum slant obtained from peritoneal exudate of Pig 1 was inoculated intraperitoneally. Dead in 12 hours. Peritoneal cavity contained a non-purulent, fibrinous exudate. Meningococci very numerous and leucocytes few. Meningococci in culture from heart's blood and exudate.

Guinea-pig 4.—Two and one-half c.c. of peritoneal exudate from Pig 3 injected into peritoneal cavity. In a few hours the animal was very sick and died in 12 hours. Peritoneal cavity contained a fibrinous exudate with many meningococci and few leucocytes. Cultures positive.

Guinea-pig 5.—One-half c.c. of peritoneal exudate from Pig 4 injected intraperitoneally. Dead in 12 hours. Peritoneal cavity contained a fibrinous exudate with many cocci and few leucocytes. Cultures positive.

Guinea-pig 6.—One-half c.c. of peritoneal exudate from Pig 5 injected intraperitoneally. Dead in 10 hours. Cultures from heart's blood and peritoneum gave meningococci. Peritoneal exudate contained few leucocytes many of which were filled with cocci.

Guinea-pig 7.—One-half c.c. of peritoneal exudate from Pig 6 injected intraperitoneally. In three hours the animal was very sick Died in 10 hours. Meningococci recovered in pure culture from heart's blood and from peritoneal exudate. Few leucocytes present.

[1] *Jour. Exp. Med.*, 1907, 9, p. 42.

[2] The guinea-pigs were all small, weighing from 250 to 300 grams.

Guinea-pig 8.—One-half c.c. of peritoneal exudate from Pig 7 injected intraperitoneally. Dead in 12 hours. Pure culture of meningococci obtained from heart's blood and peritoneal exudate.

Guinea-pig 9.—One c.c. of peritoneal exudate which had been heated to 65° for one hour[1] was injected intraperitoneally. No immediate effect. Next day animal appeared a little weak and not so lively. On second day the animal was normal.

Guinea-pig 10.—One c.c. of heart's blood from Pig 8 injected intraperitoneally. No effect.

Guinea-pig 11.—Injected intraperitoneally with one slant growth from Pig 8. Animal dead next morning. Usual findings in peritoneal cavity and heart's blood. Cultures of meningococci.

Guinea-pig 12.—Injected intraperitoneally with 4 c.c. of peritoneal exudate heated to 65° for one hour. No effect produced.

From the above experiments it is seen that four animals were inoculated successively with 0.5 c.c. of peritoneal exudate and all died in a short time of meningococcus septicemia with enormous numbers of meningococci in the peritoneal exudate. It is certain there must have been a rapid multiplication of the cocci in the animals. Furthermore, when heated sufficiently to kill the organism, the same or much larger quantities have little or no effect. There is a suggestion also of an increase of virulence, as shown by the result in Pig 2 and Pig 5. However, the experiment was not devised to show this and the result is not conclusive.

Injection of patients with killed meningococci.—Case 1.—Adult; typical case of epidemic cerebrospinal meningitis. Meningococcus was obtained from cerebrospinal fluid and nasal mucosa. Blood culture sterile. During the first few weeks the temperature varied from 100 to 104. It then became more irregular, varying from normal to 103. Patient became very emaciated. Leucocytic count at the end of the first week was 9,200; on the 11th day 10,500. During the sixth week of the disease while running the characteristic irregular temperature the patient was injected with heated organisms from the cultures obtained from the cerebrospinal fluid. The growth from several small blood-serum slants was suspended in 3 c.c. of salt solution, heated to 65° for 30 minutes and injected subcutaneously into the right arm. Six days later a similar injection was made into the left arm. No subjective symptoms of any importance occurred. Locally on the day after the first injection there was some tenderness and slight redness and swelling at the point of injection. This completely disappeared in four days. Following the second injection the local reaction was slightly more marked. A small abscess formed after a few days and 2 c.c. of sterile pus was withdrawn with a syringe. No meningococci were obtained in smear or culture. The leucocyte curve showed a prompt rise following each injection with a more gradual fall to normal in the course of about three days. The reaction was more marked following the second injection. The reaction, as manifested by the temperature curve, is hardly perceptible. Slight increase followed each injection but since the

[1] This amount of heating kills the organism.

temperature had been so variable previously, not much significance can be attributed to this rise. But it is to be noted that on the second day after the last injection the temperature fell to normal, and at no time subsequently did it rise above 98.8 F. The pulse which previously ranged from 90 to 150 gradually became more regular and soon became normal. The patient improved rapidly and in four weeks left the hospital in good condition and with no serious after-effects.

Case 11.—Adult; typical case; meningococcus obtained in pure culture from the cerebrospinal fluid. Blood cultures sterile. The temperature varied in a characteristic manner from 98 to 104. At times the patient had severe pains in the head and marked gastric disturbance manifested chiefly by severe nausea and vomiting. Opportunity was afforded to give one injection only. A 24 hours' growth was suspended in 3 c.c. of salt solution heated to 65° C. for 30 minutes and injected subcutaneously into the right arm. No subjective symptoms whatever occurred. On the following day there was a mild local reaction manifested by some tenderness, redness, and swelling. This completely disappeared in four days; no abscess formed. On the following day the temperature remained nearly normal and the leucocyte count was slightly lower. On the second day after the injection there occurred a slight rise in both the temperature and leucocyte curves. For about two weeks the patient remained in much the same condition with perhaps slight improvement. Later vomiting became more severe, she complained frequently of much pain in the head, and her general condition became worse. She died in the 10th week of the disease or five weeks after the vaccination.

The injection evidently had practically no effect.

Injection of heated meningococci into normal person.—The same organism heated in identically the same manner and in the same amount as used in Case II was injected subcutaneously in the left arm of the writer. The result was as follows: Immediately after the injection some smarting occurred locally and in a few moments became associated with a dull ache running down the arm into the hand. In 20 minutes nausea and vomiting took place. In 30 minutes occurred a severe chill lasting for half an hour, and shortly after, intense headache and muscular pain beginning in the calves of the legs and soon passing upward to all the muscles of the body. With this purging and vomiting of bile. In three hours the temperature rose to 103° F. During the remainder of the day and in the night the nausea and vomiting continued with headache, thirst, and marked prostration. On the following day there was some improvement. The nausea and vomiting subsided; temperature ran from 101 to 102° F. A diffuse rash appeared most apparent over the back; no petechia; the face was flushed; some stupor.

On the second day after the injection the rash disappeared. Slight improvement over condition on previous day. Temperature

between 99 and 100°. The urine contained large numbers of granular and a few hyaline and epithelial casts.

On the third day there developed an extensive herpetic eruption on the inner half of both left eyelids, on both lips, and on the mucous membrane inside of the mouth, chiefly over the hard palate. Tongue was heavily coated, breath offensive, and some nausea existed. Urine contained many granular casts.

On the fourth day marked improvement. Urine still contained a few casts and trace of albumin. From this time on gradual improvement occurred with no complications of any kind.

At the site of injection there was some redness, swelling, and tenderness for three or four days. No abscess formed and at no time were the axillary glands enlarged.

The temperature, leucocyte, and opsonic curves are given in Chart 1.

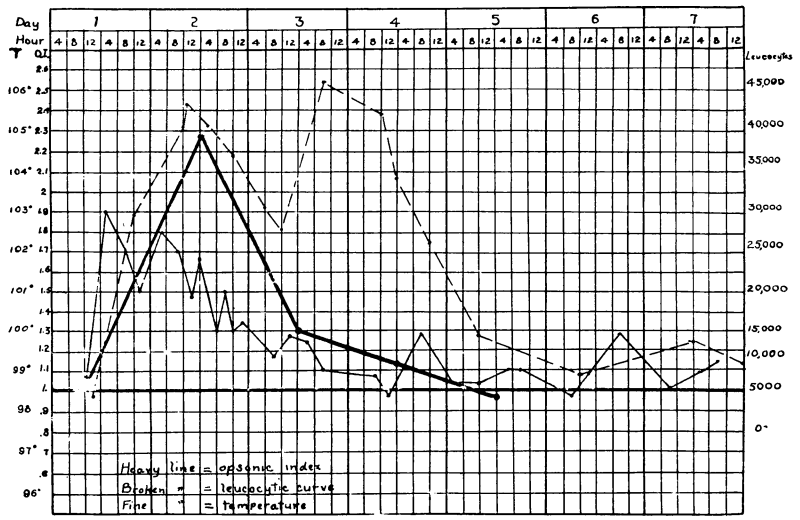

CHART 1.—Opsonic, Leucocytic, and Temperature Curves Following the Injection into a Normal Person of Heated Meningococci.

The temperature promptly rose to 103 and then followed a rather gradual decline, reaching normal on the morning of the fourth day. For nearly a month there was a slight evening temperature amounting to about one degree. With this there was some headache and slight indisposition.

The leucocyte curve also rose promptly. The maximum was reached on the third day when the white cells numbered 44,050. They rapidly disappeared and reached normal on the fifth day.

The opsonic index was determined each day. The organism was the same as that injected. Falling quantities of serum were employed, and in the preparation of the curve the sum total of all the cocci taken up in all the dilutions was used. A similar but not so uniform a curve may be obtained if instead of the sum total, any one of the dilutions is used. The character of this curve is very similar to the leucocyte curve. The rise is prompt and the decline is more gradual, the normal being reached on the fifth day. There was no negative phase unless it should have occurred between the time of the injection and the time the index was determined on the following day.

There was here a prompt and marked reaction in a normal individual from the subcutaneous injection of dead meningococci. The symptoms were those of a profound toxemia and no special meningeal symptoms occurred except those attributable to a general toxemia involving the central nervous system. The meningococcal substance is readily diffusible, as indicated by the very prompt reaction; this occurring in 20 minutes. It affected especially the nervous system because of the violent headache, slight delirium, and vomiting. Of interest is the extensive herpes, pointing most probably to an involvement of the Gasserian ganglia, which is in accord with the frequency of herpes in the disease as it occurs naturally. There was produced a marked acute nephritis. No focal symptoms occurred.

There was also a sudden reaction on the part of the protecting mechanisms of the body as shown by the temperature, leucocytic, and opsonic curves. The close correspondence in the rise and fall of the curves is very striking. The leucocytes increased in numbers about eight times and the opsonin more than doubled in amount according to the curve. The sum total of this increase would therefore be highly significant.

As already stated the organism and the amount injected in the normal individual were identical with that used in one of the meningitis cases. The results are strikingly different. The most probable explanation is that the individual with meningitis had acquired a marked immunity and the relatively large number of cocci had

almost no effect. When one considers the large amount of spinal fluid and the large number of meningococci often present not only in the fluid but in the blood and other parts of such patients, the number of organisms injected in these cases must be quite insignificant; consequently the effect in the patient need not be marked.

One must be extremely cautious in drawing any sweeping conclusions from such experiments. It is quite possible, indeed probable, that normal individuals vary in their susceptibility to the effect of meningococci and the same amount injected into another normal individual might produce possibly milder, possibly more severe symptoms. Pointing in this direction is the variation in the meningococcidal property of normal bloods already discussed. The variation of the virulence of the organism is another factor of great importance and must be considered in every instance. Again animal experiments must not be used as reliable indicators of the possible results upon human beings. What may have practically no effect upon a rabbit may cause profound toxemia in man, as was true in this instance.

The above data are entirely inadequate to make any definite statement concerning the value of killed meningococci in meningitis. The following suggestion may be made: In the early stages—during the first week—the organism has established very little if any immunity,[1] consequently only very minute amounts of killed cocci should be used at this time. From this time on increasing doses may be given watching the temperature, leucocyte and opsonic curves from day to day. The above curves suggest that injections might be made every 6 to 8 days. So many cases of this form of meningitis run a protracted subacute or chronic course, the temperature variations being very marked even for weeks, and often resulting in some irreparable deformity. Such cases, it would seem offer the greatest inducement for therapeutic inoculations.

NOTE.—Since this article was written several papers have appeared on the subject. Houston & Rankin (*Lancet*, 1907, 172, p. 1213) report upon the opsonic findings in 63 cases of cerebrospinal fever. From the sixth day on, all the cases showed an opsonic index of over 4.0; in some cases the index was very much higher. They call attention to its value in diagnosis. Von Eberts and Hill (*Am. Jour. Med. Sci.*, 1907, 134, p. 35) inoculated three cases of cerebrospinal fever with heated meningococci.

[1] The reaction of agglutination appears about the end of the first week.

Opsonic determinations showed a considerable increase following the inoculation. Two cases recovered and a third terminated fatally. Birnie and Smith (*Am. Jour. Med. Sci.*, 1907, 134, p. 582) report a case which showed an increase in the opsonic index following the injection of a meningococcus vaccine.

In the above cases treated with the vaccine the question arises as to how much the increase in the opsonin is due to the injection of the dead cocci and how much to the increase that naturally occurs in the course of the disease as shown by the observations of Houston and Rankin.